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Altitudinal adaptation of *Arabidopsis*
Adaptace *Arabidopsis* podél výškového gradientu

Bachelor´s thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 15. 8. 2018 Doubravka Požárová

Abstract

Study of adaptation provides insights into a key part of evolutionary processes. This thesis presents an overview of an adaptation process and further focuses on local adaptation – a process of selection of phenotypes and underlying genotypes that fits the best to a particular environmental condition. Then, the thesis provides a brief summary of methodical approaches how to detect local adaptation, on both, molecular and phenotypic level. Further I focus on alpine environment as a model to study local adaptation, due to complex set of stresses imposing strong selection pressure, their variable strength structured along altitudinal gradient and island-like distribution triggering parallel origins of locally adapted ecotypes. Such is also the case of local adaptation to mountain environment in plant model genus Arabidopsis. I here review the responses to environmental variables along altitudinal gradients and synthesize the environmental factors that are involved in the local adaptation process: precipitation, water regime, temperature and radiation. The recorded adaptive traits were associated mainly with life history, phenology, growth forms and drought stress tolerance. Study of alpine adaptation helps with understanding evolutionary and ecological processes and it is also important to assess how plants (endangered species in mountains, but also the field crops) will respond to warming or drought in changing environment.

Key words: Local adaptation, Arabidopsis, Alpine environment, adaptive traits, environmental conditions

Abstrakt

Studium adaptace umožňuje vhled do klíčové části evolučních procesů. Tato práce představuje zakladní informace o adaptačních procesech a dále se zaměřuje na lokální adaptaci. Během lokální adaptace dochází k výběru fenotypů (a genotypů, ze kterých jsou odvozeny) nejlépe přizpůsobených konkrétnímu prostředí. Poté se práce zaměřuje na stručný přehled metodických přístupů k detekci lokální adaptace na molekulární i fenotypové úrovni. Dále se zaměřuji na alpské prostředí. Díky komplexnímu souboru stresových faktorů, působících selektivně a s různou intenzitou podél výškového gradientu a ostrůvkovité distribuci (umožňující paralelní původ lokálně přizpůsobených ekotypů) je horské prostředí ideálním modelem pro studium lokální adaptace. Tak he tomu i u lokální adaptace na horské prostředí u modelových rostlin rodu *Arabidopsis*. Uvádím odezvy studovaných rostlin na proměnné vlastnosti prostředí podél výškového gradientu a shrnuji faktory, které se podílejí na lokální adaptaci: srážky, vodní režim, teplotu a záření. Zaznamenané adaptivní znaky jsou spojeny zejména s životním cyklem, fenologií, růstovými formami a tolerancí stresu vůči suchu. Studium adaptace ve vysokohorském prostředí pomáhá porozumět evolučním a ekologickým procesům, a také tomu, jak rostliny (ohrožené druhy v horách, ale i polní plodiny) reagují na oteplování či sucho v měnícím se prostředí.

Klíčová slova: lokální adaptace, Arabidopsis, vysokohorské prostředí, adaptivní vlastnosti, podmínky prostředí

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Contents

1 Introduction

Evolution of organisms, resulting in precisely match between organism and its' environment, is fascinating process. The adaptive traits allow plants and animals to use variable resources and survive in different conditions. Study of local adaptation provides information about evolutionary processes and ecosystem behaviour. It also provides clue how plants, sessile organisms, can respond to environmental changes and colonize novel areas.

In this thesis, the adaptation of plants along altitudinal gradient, especially to high elevations will be reviewed. High altitudes provide useful model for local adaptation studies. Island like distribution allows independent adaptations of different populations. Complex set of climatic, topographic and other variables represent strong and detectable selective pressure. *Arabidopsis* is an ideal model for studying adaptation, thanks to large amount of knowledge about this genus.

Methods of detection local adaptation are presented in further part. Local adaptations are detected on different levels. High fitness of specific phenotype and traits in comparison with other phenotypes in environment is sign of adaptation. Selective pressure and adaptability of some genes (resulting in traits) could be recognize also on molecular level from molecular and genomic data.

Evidences of alpine adaptation, adaptive traits (life history, phenology, growth forms and drought stress tolerance) and the crucial triggers (water regime/precipitation, temperature, radiation), which were detected in studies are summarized.

The main aims of this works are (1) brief introduction to adaptation, its types and causes, highlighting the specific aspects of plant biology, (2) review of methodological approaches used in study of local adaptation, (3) synthesis of studies focused on adaptation associated with altitude in *Arabidopsis* and related species within the Brassicaceae family.

2 Adaptation

2.1 Adaptation and Natural Selection

Adaptation in a broad term is the ability to suit better to particular conditions. Each organism on the Earth has to interact with its natural environment which varies in space and time. Living creatures are not independent units, they face diverse abiotic and biotic factors to which they have to adapt.

The process of adaptation is not a targeted movement to ideal form. It is enabled by heredity of genetic information, which contributes to the phenotype of an organism (Watson and Crick, 1953) Morphological and physiological traits, behaviour or development are derived by expression of genetic information contained in cells. Genetic information is variable among individuals, as the shapes, sizes, behaviours or colours are.

Some individuals with specific phenotype or behaviour are able to survive better and breed more in certain conditions than do the others. This mechanism, known as natural selection, drives the adaptation process. Differences in survival are, however, often not the major outcome of the selection process. Instead, the number of offspring and their further evolutionary success, rated as fitness, is the key variable in the adaptation process. Genetic information passes from parents to offspring and concrete alleles which contribute to the larger reproductive output then spread through population. Genetically based functional properties of organisms which allows them to reproduce more than the others are named adaptations (Fisher, 1958). The process of adaptation can be thus viewed as a sequence of changes of allelic frequency and trait values in population through time. These changes lead to higher frequency of better environmentally matched phenotypes in population (Howlett and Majerus, 1987).

Notably, not all changes in phenotypes in population are sign of adaptation. Some traits could be non-adaptive, they can become widespread thanks to selection of close genetic regions or they can be connected and affected by other (adaptive) traits or it could be result of stochastic process.

The natural selection process could work only in genetically variable populations. There are several processes how genetic variation of a population is generated. Primary source of variation are mutations, spanning from single nucleotide variations to larger insertions and deletions to alternation of chromosome

structure and whole genome duplication. New combinations of alleles then arise during recombination in meiosis and gamete combination in sexually reproducing species. At a level of multiple populations, migration and introgression also ensures further input of novel genetic variants.

2.2 Polyploidy

Polyploidy is a large scale mutation that is present across all eukaryotes (Ohno, 1967), but with particularly high incidence among plants (Otto and Whitton, 2000). Multiplication of whole set of chromosomes plays role in speciation and diversification of lineages (Petit and Thompson, 1999), but the consequences of polyploidy for evolution and speciation are usually not straightforward and thus still poorly known.

Two major types of polyploidy in plants are distinguished. Autopolyploid lineages have two copies of chromosome sets derived from only one species. Allopolyploids combine genomes of two different (related) species, therefore they reflect both the effect of multiple gene dosage (polyploidy) and the coexistence of multiple distinct (sub)genomes (hybridity). In addition to changes in structure and genes resulting from genome doubling, allopolyploids may also confer new beneficial interaction of genes of distinct origin.

The adaptive value of polyploidisation is not taken as a general rule. Contribution of polyploidy to better adaptability is subject of an ongoing debate (Weiss-Schneeweiss *et al.*, 2013) similarly as are the mechanisms potentially conferring such advantages. Possibility of evolving novel functions of genes thanks to higher number of mutational targets, redundancy of copies, faster mutational rate induced by instability of new genome (Wendel, 2000) (Sémon and Wolfe, 2007) and phenotypic changes caused directly by the larger genome are considered as advantages of polyploidy (Ramsey, 2011). It has also been empirically documented, that polyploid yeasts could undergo faster adaptation than haploids or diploids (Selmecki *et al.*, 2015). Mutational rate or tendency and ability to survive with some genetic abnormalities, as polyploidy, could be then itself an adaptive trait.

2.3 Phenotypic plasticity

Genetic background is not the single factor affecting the phenotype. Some traits are environmentally modelled, depending for example on diet, temperature or water accessibility. Phenotypic plasticity is the ability of genetically close (or even identical)

individuals to undergo phenotypic alterations in response to the variable environment (Bradshaw 1965).

A single genotype could develop into different phenotypes in reaction to different conditions solely due to resource or spatial limitations or non-adaptive reactions in physiological processes. Some changes are just passive responses towards the environmental triggers but in other traits the tendency towards larger plasticity could be under a genetic control and as such could be selected for when beneficial.

Such case of genetically conditioned adaptive phenotypic plasticity is assumed as a way how to improve survival chances in changing and unpredictable environment (Coquillard, Muzy and Diener, 2012) (Masel, King and Maughan, 2007). Phenotypic plasticity guarantees faster response than the process of adaptation as it can affect phenotype immediately or in shorter time scale. On the other hand, such ability to undergo phenotypic switching could limit the rates of adaptation as the genotypes of highly plastic organisms are less 'visible' for selection.

2.4 Local adaptation

Local adaptation is a process in which populations accommodate better to a particular environment. This is enabled by acquisition of a set of traits which provide fitness advantage under such environmental setup. These advantages provide the resident genotype the ability to better survive and have more offspring than the other, less well adapted genotypes from the same or other population(s). When growing in its native environment, fitness (relative reproductive success) of the locally adapted population is higher than fitness of other populations originating from different environments. On the other hand, when such population is transplanted to different environments, its fitness decreases implying that locally adapted population has the highest fitness in its native habitat (Williams 1966) (Savolainen, Lascoux and Merilä, 2013).

There are, however, several forces that operate against local adaptation and may ultimately lead to failure of the entire adaptation process. Firstly, natural selection usually operates in inter-connected networks of populations, each of which grows in (slightly) distinct environment. Such environmental variation could be structured among relatively isolated places or along some environmental gradients. Connected populations are affected by gene flow which homogenises the differences

acquired during local adaptation. To keep the set of locally beneficial traits, the effect of selection has to be stronger than the homogenizing effect of migration. Secondly, strong phenotypic plasticity can weaken the phenotype-genotype link and thus reduce the effect of selection. Thirdly, temporally instable directionality of selection may not allow adaptation towards particular set of factors (Kawecki and Ebert, 2004) Finally, in small populations genetic drift also reduces strength of selection and consequently limits the ability of adaptation (Wright, 1931). Occurrence and intensity of local adaptation depends on certain properties of species or population such as phenotypic plasticity, migration potential or standing genetic variability and it also depends on population structure, connectivity and size.

Characteristic genotype (and its phenotype) associated with certain environment is defined as ecotype (Turesson, 1922). A specific phenotype is established due to habitat – species interactions leading to accumulation of set of heritable locally specific traits, i.e., the local adaptation process. The set of characters is not acquired by chance and nearly similar ecotypes can evolve independently and repeatedly in different areas characterized by similar ecological conditions (Turesson 1925). The concept of ecotype is complicated by existence of intermediate phenotypes (either due to underlying ecological gradient or later hybridization between the ecotypes) and impossibility of distinguishing groups along environmental gradients where the continual changes are observed (Bradshaw 1959). However, in certain cases, when reproduction barriers arise between the ecotypes, the two units may differentiate into a separate species in a process called ecological speciation (Nosil and Crespi, 2004). Studying of different ecotypes thus significantly contributes to understanding of evolution and variation including the origin of species.

2.5 Consequences of adaptation

Adaptation is one of the key processes that generate the fascinating range of diversity that surrounds us, contributing to variability and complexity of life. Further insight into adaptation can help us to understand the processes behind the diversity on the Earth and to protect this unrepeatable variability.

Fast and efficient adaptation is particularly important for sedentary organisms, such as plants. Immobile organisms with limited migration capacity (slow or without precise direction) cannot easily leave places with unfavourable conditions and thus they need to react towards the changing environment in a different way. Besides

phenotypic plasticity, allowing fast response to immediate changes, local adaptation is the key process that allows plant populations to survive ongoing changes of environment and/or colonise new habitats (Savolainen, Lascoux and Merilä, 2013).

Studying of local adaptation, its underlying mechanisms, and rate of changes have also some broad implications for human civilization. By knowing the triggers and consequences of the process, we can better predict how the climatic changes, global warming, and human impact will affect the plant populations and entire habitats (Jump and Peñuelas, 2005) (Doak and Morris, 2010). Assuming the risk of extinction of species based on population dynamics and adaptational potential is important in nature conservation (Trouwborst, 2009). Questions connected with adaptive abilities of plats are also ubiquitous in agriculture and forestry, such as the benefits of using locally adapted strains in timber cultivation. Capability to grow food crops in changing conditions (Matthews *et al.*, 1997), especially in warm and drought is a key challenge for current society. Understanding of biological processes that may stand behind may help to handle its application more efficiently.

3 Alpine adaptation

3.1 Alpine environments

Alpine environment, characterised by harsh and unpredictable conditions, is one of the major challenge triggering local adaptation in plants. Plants in high altitudes are limited by entire set of factors including short growing season, low and/or fluctuating temperatures, freezing, snow accumulation, strong wind and other factors limits the growth, seed production and surviving. The complex set of climatic factors differ among mountain ranges and it is hard to define alpine environment in general. The boundaries of alpine environment varies in altitude widely (from sea level in arctic regions to several thousand metres in tropical mountains) and are not clear in some cases with specific local topography or long history of human land use (Treml, Jankovská and Petr, 2006). It is limited by timberline, in most cases, but also other factors, can limit the distribution of trees and type of vegetation.

Interestingly, latitudinal gradients change to some extent in a similar manner as the altitude does, in particular with respect to temperature and climatic severity. The arctic and mountain tundra also shows some similarities. The overlap in species is present especially in arctic regions and high mountains in temperate and cold climate. The crucial difference between circumpolar and alpine areas is, however,

within-year variation in a day length and solar radiation limitation during year in polar areas.

Species diversity in high altitudes has the following three major sources. Some species are relicts from colder periods and ice ages. They have discontinuous distribution, frequently occurring both in arctic and alpine areas. Second group of species evolved more-or-less *in situ* in the mountains. Plants can colonize the mountains from lower altitudes and evolve under the novel stressful conditions into a new ecotype. In case hybridization barriers arise between the foothill and alpine ecotypes, a new species is formed Another evolutionary process is connected with the mountain orogeny. During uplift, environmental conditions change and only the adapted plants survive and potentially further turn into a new species.

Alpine habitats are characteristic by spatially isolated, island-like distribution. Such pattern triggers repeated evolution of similar traits in distinct mountainous areas. Parallel processes in set of conditions allow to compare among replicates (mountain ranges) and to discriminate general rules of adaptation from idiosyncratic processes operating in a different way in each mountain range.

The global climatic changes present possible risks for alpine environment. Shifts in ranges happen in real time, and the rate of change is highest in polar regions as well as in high mountains. Limited areas of ridges and peaks and island distribution of mountains do not provide the opportunity to migration. It presents threat for narrow endemics in mountains. Adaptation or extinction are then the other possibilities for alpine species in changing world (Crawford, 2008).

4 How to detect local adaptation

Adaptation has a phenotypic manifestation, demonstration of selected genotype in specific conditions. Signatures of adaptation are then detectable at different levels, genotypic and phenotypic. Comprehensive view of the adaptation process is, however, gained only by connecting the data about genes responsible for the adaptive traits and phenotype of plants in different environments (translating into differences in fitness). Thus, a complex set of experiments is typically needed to reliable detect adaptive traits and their underlying genetic basis (Gompert *et al.*, 2014) (Barrett and Hoekstra, 2011).

4.1 Common garden and transplant experiments

One possible way how to prove local adaptation is reciprocal transplant experiment.

The plants from one population are transplanted into a habitat occupied by a different population (and vice versa) and fitness of the native and non-native population is compared at each site. Locally adapted population shall exhibit higher fitness in its native environment as compared to performance of the non-native 'reference' population. Plants are exposed to complex set of natural conditions, which are not possible to set in laboratory conditions, but such approach also brings some disadvantages. Transplant experiment is extremely technically hard and time-consuming, what limits the number of populations and plants in experiment and thus its power for generalization across populations and species. Transplantation of seedlings instead of seeds reduce our knowledge about the role of germination processes and early development stages in adaptation. Differences among long-lived and slow growing plants could be detectable after a very long period Last but not least, the ethical problems are connected with this type of experiments, because of foreign genotypes are introduced into environment.

An alternative strategy to detect the differences in heritable traits among plants from different populations is letting them grow in the same, standardized conditions. In such common garden experiments it is possible to detect fixed phenotypic changes and differences, because of elimination of factors which lead to phenotypic variability, for example water limitation or intensive disturbance. It is also possible to manipulate a limited a set of controllable factors, as a light or temperature (for example in growth chambers) and investigate responses of plants with different origin. Common garden experiment, however, has only limited power to detect local adaptation as the environment used is usually different from that occupied by either of the target populations.

4.2 QTL – mapping and association mapping

The method which allows to connect the adaptive phenotype with candidate regions in the genome is a quantitative locus trait mapping – QTL. A segregating F2 population is created by crossing lineages with different origin (e.g. differentially locally adapted populations) and further selfing or cross of such hybrid F1 plants. Each offspring in the resulting F2 mapping population has a different combination of traits and underlying alleles inherited from both parents. Different combinations are created due to meiotic processes. Fitness, phenotype and molecular markers representatively covering entire genome (e.g. reduced representation libraries such as

genotyping-by-sequencing) are then associated in large number of individuals and genes responsible for adaptive traits are found.

The phenotype – genotype relation is used also in association mapping. Natural populations are sampled, genetically and phenotypically. These two data sets are then associated over large number of samples and statistically significant association is taken as a signal for genetic basis of a particular trait. As in other population genetic based methods, an impact of genetic structure and population history can cause a problem as neutral genetic structure may provide false positive sign (Rellstab *et al.*, 2015).

4.3 Molecular tools

Signs of adaptation are also detectable at a genome level because the variation and structure of a genome is influenced by selection processes. Selected beneficial alleles spread through population because they provide advantages to their owners. Because not genes, but entire chromosomal regions are functional units inherited during meiosis surrounding physically linked regions are also swept by selection. The result is relative genetic uniformity of regions physically linked with genes under selection. Other parts of genomes are older and has a tome to mutate in different forms. Selective sweeps are then detectable by different mathematical tools in whole genome sequences or data which contains information about polymorphism. Then the candidate genes are estimated through contrasting against neutral genetic structure and their functions could be associated with conditions and traits under selection. This method has become more common recently with progress in high-throughput sequencing.

5 Arabidopsis as a model taxon

Genus *Arabidopsis*, a part of Brassicaceae family, includes nine species widespread in temperate regions of northern hemisphere. The most known species is thale crees (*Arabidopsis thalina*), which is one of the most important model species in plant physiology and genetics. Thanks to relatively small genome size, easy cultivation (fast life cycle, high amount of seeds etc.) it is ideal for research of plant biology.

Other relative species, as *Arabidopsis lyrata, Arabidopsis halleri* or *Arabidopsis arenosa*, have emerged as model species recently. The function of their genes and physiology are better known than in other species thanks to long research on *A. thaliana*. Unlike *A. thaliana*, these species are outcrossing thus providing large

variation both for quick adaptation response in nature and also for precise detection of causal molecular variation underlying adaptation (Yant and Bomblies, 2017). In addition, ecological niche of all these species is very wide and they face distinct environmental stresses such as heavy metals (*A. halleri*) (Talke, 2006), arctic stress and small populations (*A. lyrata*) (Paccard, Fruleux and Willi, 2014) and alpine conditions (*A. arenosa*) (Kolář *et al.*, 2016) These species thus allow address different evolutionary and population genetics questions then can research on Arabidopsis thalina reply.

For example, *Arabidopsis arenosa* is outcrossing species, with large natural variation of both alleles and phenotypes that also encompasses natural populations of autopolyploids. It also exhibits large variation in life history from (nearly) annual ecotypes growing on railway ballast to strictly iteroparous plants growing in high altitudes. It is ideal species for studying alpine adaptation, because of independent colonization of high altitudes by genetically distinct lineages in at least four mountain ranges in Europe.

6 Triggers and their responses

From both, ecological and evolutionary point of view, one of the most interesting question is to associate adaptive phenotypic responses of plants to specific environmental factors and detect genetic basis underlying these traits. Different studies are focused on different part of this problem in alpine adaptation, there is, however, little synthesis on this topic going across species and mountain ranges.

Altitudinal gradient is characteristic by a whole set of gradually changing climatic (temperature, precipitations), topographic (slopes), soil associated (rocks and scree) and biotic (pollinators, herbivores, competition) factors. Not all of them could be easily separated from each other. Equally in most of the studies more variables than altitude is addressed. Especially the confounding effect of latitude and climatic conditions (on a larger scale) is frequent and hardly eliminated.

The temperature and precipitation was founded as the crucial climatic variables for adaptation of some plants in the mountains (Manel *et al.*, 2012) (Kubota *et al.*, 2015). Lot of other climatic variables influencing plant growth, for example presence and thickness of snow cover (Molau, 2018), are connected with temperature and precipitation. Further abiotic conditions, which have been detected in some studies are radiation (Fischer *et al.*, 2013).

Although the phenomenon of alpine adaptation is widely widespread in plants (Halbritter *et al.*, 2018) our information of its influence on traits and their genetic basis is rather fragmentary, separated into specific case studies. Alpine ecotypes share some general similarities such as shorter individuals and less production of biomass (Halbritter *et al.*, 2018). But all trends and mechanisms of alpine adaptation are not clearly understood yet, even in relatively well-known species and genera such as *Arabidopsis*.

6.1 Unpredictable conditions

Life-history strategies, such as perenniality or annuality and relative investment into generative and vegetative parts strongly vary according to the stressful conditions. A large transplant study of *Arabidopsis lyrata* documents the large difference in survival of local a nonlocal population at a high-elevation site suggesting strong local adaptation. Whereas most of the individuals from the lower site died after the first year, the alpine-originated individuals plants survived. They produced less flowers than the lowland ones, but they were able to produce them for more than one season suggesting trade-off into annual reproductive investment (Leinonen, Remington and Savolainen, 2011). Investment into more than one vegetative rosette is functional strategy also for *Erysimum capitatum* in alpine conditions (Kim and Donohue, 2013).

The risk of reproductive investment into only one flowering occasion is probably influenced by overall shorter growth season, high risk of summer frost or long period of low temperatures, during which the flowering could not be successful. For a plant which lives more than one year, it is important to measure fitness as a sum of fitness over years, data from the measurement of only the first year could lead to misinterpretation.

Measuring of rosette size, total biomass and roots are commonly used as a proxy of fitness, also reflects the ability of plant to invest into vegetative phase.

6.2 Short growing season

The growing season in high mountains is limited by low temperature and long persisting snow cover. The precise timing of developing is then necessary to utilise limited time (Lütz, 2013). In northern regions, the growing season is also strictly limited by day length and photoperiod. Early flowering higher and nothern site was detected in *Arabidopsis lyrata* (Leinonen *et al.*, 2013).

6.3 Herbivores and pollinators

Altitude influences also animals and then the impact of altitude could be mediated by them. For example, herbivory by large herbivores (sheep) is strong selective pressure against large inflorescences in alpine populations of *Arabidopsis lyrata* in Scandinavia, because such large inflorescences attract the herbivores. The number of inflorescences is then negatively correlated with reproductive output in high elevations (Sandring *et al.*, 2007), because conspicuous plants are damaged by grazing more frequently and small inflorescences are selected. Potentially less human-mediated is the effect of insect herbivory. It was measured in *Boechera stricta* in Colorado mountains and in some years, the impact of elevation of origin of the source population on herbivory was significant. In low altitudes, the native plants were more resistant and plants from high altitudes were more damaged (Anderson *et al.*, 2015).

The floral display and flowering period of a population can be also selected by pollinators. The usual direction of such pollinator-mediated selection goes towards increase in number of flowers and size of petals – more attractive for pollinators. The effect of pollinators also induces later start and early end of flowering, because in the end/start of flowering season the probability of pollinating decrease. *Arabidopsis lyrata* is predominantly outcrossing species. It is dependent on insect pollinators, which then represent an additional selective pressure especially in cases when pollen limitation is present (Sandring and Ågren, 2009). The species composition of pollinators is changing along altitudinal gradient, then the selection is mediated by them (Hodkinson, 2005) and it is expectable also in other species. One of the problems in such approach is that we measure only female reproductive success in most of cases.

6.4 Temperature

Temperature stresses includes freezing temperatures (Zuther *et al.*, 2012) and low temperatures during year (Fischer *et al.*, 2013) which represent problems for plant surviving.

Combination of wind and low temperatures is probably the reason for characteristic short, nearly cushion, plants in alpine conditions were observed in Arabidopsis also. The molecular mechanisms leading to short growth was studied in Arabidopsis thaliana in the Alps. During the transplant experiment it was proved,

that this trait is adaptive and the genetic basis of this adaptation in A. thaliana was found. Genetic basis of this mutation is a single nucleotide mutation in gibberellin signalling pathway (Luo *et al.*, 2015).

6.5 Water regime

Drought stress means the problems with accessible water, in winter, one of the biggest problems in plants is osmotic stress, induced in cells by dehydration. The water cannot stay in the cells, because of risk of ice crystal development.

Adaptive differences in growth forms was also observed in *Erysimum cappitatum*, where the production of multiple rosettes is characteristic in populations from high altitudes (Kim and Donohue, 2013). The defined factor for this differentiation is drought stress.

6.6 Radiation

Genes involved in light reaction pathways were identified as genes under selection in alpine populations of *A. lyrata*. Hypocotyl length response to light and potential part of light signalling transduction were traits underlying by the candidate genes (Fischer *et al.*, 2013).

7 Conclusions

Different adaptive traits, including life history, phenology, growth forms, drought stress tolerance and freezing stress tolerance response to the climatically triggers: water regime/precipitation, temperature and radiation were observed in the studies.

Combination of different methods seem to be necessary to prove adaptability of some traits and genes. And to understand phenotype – genotype connection. Physiological measurements are necessary to elucidate mechanisms of adaptations.

Long duration of experiment it is important to detect adaptive advantages of native populations, because of variation among years and longevity of some plants, where the fitness is a sum of fitness from more than one year.

Many studies are concentred at whole set of local environmental conditions, including altitude, latitude, water regime, temperature. In such a type of design, it is not possible to clearly determine which variable has the crucial role or if the combination is important factor.

8 Further studies

To expand our knowledge on alpine adaptation in *Arabidopsis* I focused on adaptation of multiple independently formed alpine ecotypes of *Arabidopsis arenosa*. I specifically asked following questions that will represent the basis of my following master thesis: (1) Which traits are crucial for plants in high altitudes (allow them to survive there)? (2) What is the ratio of phenotypic plasticity to heritability? (3) Which mechnisms (on molecular level) are important in adaptation of plants?

Building on unique design of multiple (four) pairs of foothill and alpine populations from distinct areas of the Alps and Carpathians, we established a common garden experiment in growth chambers. In addition to cultivation all populations of distinct ecotype (foothill/alpine) and mountain region in one chamber, we also manipulated two crucial parameters that affect plant life in high altitudes - temperature and irradiance – in a full-factorial design.

Two foothill-alpine population pairs from three mountain ranges (Eastern Alps, Tatry and Fagaras) were included, with the duplication of diploid and tetraploid populations in Tatry. These alpine ecotypes were formed independently. We wanted to estimate which portion of morphological and phenological variation in populations is caused by phenotypic plasticity and which instead by genetic background.

The preliminary data indicate that the overall appearance remains different between the alpine and foothill populations when exposed to the same conditions. On the other hand, some traits are strongly modulated by the experimental conditions and the level of phenotypic plasticity differs across treatments, altitudes and regions of origin.

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